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1 **Title**

2 New soil, old plants, and ubiquitous microbes: Evaluating the potential of incipient basaltic soil
3 to support native plant growth and influence belowground soil microbial community
4 composition

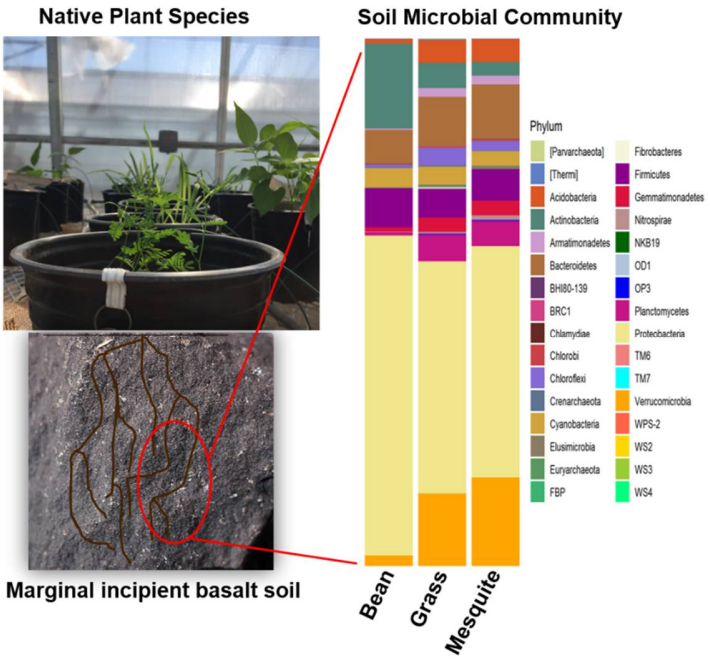
5

6 **Abstract**

7 The plant-microbe-soil nexus is critical in maintaining biogeochemical balance of the
8 biosphere. However, soil loss and land degradation are occurring at alarmingly high rates, with
9 soil loss exceeding soil formation rates. This necessitates evaluating marginal soils for their
10 capacity to support and sustain plant growth. In a greenhouse study, we evaluated the capacity of
11 marginal incipient basaltic parent material to support native plant growth, and the associated
12 variation in soil microbial community dynamics. Three plant species, native to the Southwestern
13 Arizona-Sonora region were tested with three soil treatments including basaltic parent material,
14 parent material amended with 20% compost, and potting soil. The parent material with and without
15 compost supported germination and growth of all the plant species, though germination was lower
16 than the potting soil. A 16S rRNA amplicon sequencing approach showed *Proteobacteria* to be
17 the most abundant phyla in both parent material and potting soil, followed by *Actinobacteria*.
18 Microbial community composition had strong correlations with soil characteristics but not plant
19 attributes within a given soil material. Predictive functional potential capacity of the communities
20 revealed chemoheterotrophy as the most abundant metabolism within the parent material, while
21 photoheterotrophy and anoxygenic photoautotrophy were prevalent in the potting soil. These
22 results show that marginal incipient basaltic soil has the ability to support native plant species
23 growth, and non-linear associations may exist between plant-marginal soil-microbial interactions.

Keywords: marginal soil, basalt material, land degradation, native plant species, microbial community

Graphical Abstract



Authors

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1. Introduction

Soils provide a wide range of ecosystem services and are central to sustaining life in the biosphere. These include supporting plant growth and sustenance, ensuring food security, and modulating biogeochemical cycles. Additionally, soils hold anthropological significance as civilizations have developed and flourished around their ability to harness soils' power to grow crops. However, global soil loss is occurring at unprecedented rates with depletion rates twenty times faster than formation in the United States alone [1,2]. Soil loss and eventual land degradation is exacerbated by anthropogenic activities including urbanization, population growth, machine-intensive agricultural practices, conversion of forest land to agriculture land, and land-use practices such as mining. Degraded land quality, in turn, negatively impacts food production, livelihoods, and ecosystem services [3].

The process of rock to soil formation and therefore, landscape development, occurs over hundreds of years, with a predicted 500-1000 years needed to form one inch of top soil [4]. This imbalance between rates of soil formation and depletion is unsustainable. Soil conservation therefore stands out as a necessity if humans are to secure food, fiber, economy, and health. The Land Degradation Neutrality (LDN) Program adopted by the United Nations Convention to Combat Desertification (UNCCD) recognized that land conservation requires restoring degraded land and soil to achieve "degradation-neutral world" [5]. Within the UNCCD framework, land degradation neutrality is defined as balancing land degradation (losses) with conservation and restoration efforts (gains). A key concept of the neutrality framework is to improve the productive potential of land/soil that is already degraded [6], with calls for restoration and rehabilitation mechanisms. We propose exploring the capacity of marginal soils as one approach to increase ecosystem service, function, and productivity of degraded lands.

Marginal soils can include incipient soils, soils affected by mining processes and fire, over-used agricultural lands, urban vacant plots, and primary succession ecosystems [7,8]. Soils in these landscapes have persistent lack of plant growth and are characterized by lack of stable soil aggregates, organic carbon forms, and essential plant nutrients [8]. Rather than having a use-and-throw perception of soil, where soil is abandoned after it deteriorates in quality, it is beneficial to evaluate the capacity of marginal soils to support plant growth. While not all marginal soils will be suitable to support food crops, soils that are able to support plant cover will prove beneficial for natural ecosystems in general. For example, vegetated marginal lands can reduce soil erosion, stabilize soil structure, and reduce precipitation run-off. Effective vegetation establishment on such marginal soils may benefit from sowing native seeds that are adapted to local climatic regimes and soil types [9]. Moreover, the use of native seeds best suited to local soil and climate [10] can promote crop and biodiversity conservation, and preserve indigenous knowledge and heritage.

Land degradation reduces soil microbial biomass and microbial activity [11], with reports of significant decrease in beneficial microorganisms and increase in pathogenic ones in degraded soils [12]. Another study [13] found higher bacterial richness and diversity in restored soils and soils under native vegetation in comparison to degraded soils. As invisible engineers of terrestrial ecosystem, soil microorganisms contribute to soil structure, are involved in biogeochemical cycling of nutrients [14], decompose organic matter, impact plant diversity and productivity, and play a critical role in soil fertility [15]. The aboveground-belowground links between plant and microbes are especially crucial in marginal systems characteristic of nutrient-poor soils and inferior soil structure. Soil biota is one of the five soil forming factors proposed by Hans Jenny

[16]. In marginal soils lacking higher life forms, microbes therefore are the first biotic component to contribute to soil stabilization and structure. Hence, evaluating microbial community establishment and response in marginal soils is critical. Empirical knowledge of these linkages can then be used to enhance marginal soils' capacity to support plant growth, for e.g. by developing microbe-mediated amendment technologies suited to low productivity landscapes.

This study evaluated the capacity of basaltic soil material to support native plant growth and the associated variation in soil microbial community dynamics following a month-long greenhouse experiment. The incipient soil is basaltic crushed tephra sourced from Merriam crater in northern Arizona and is being extensively studied at the Landscape Evolution Observatory (LEO) housed at Biosphere 2 in University of Arizona, to understand coupled hydrobiogeochemical processes of landscape evolution [17,18]. This lithogenic basalt parent material is oligotrophic, with low carbon content (0.001 ug/mg) [19] but has been shown to harbor microbial life [20]. The objectives of this study were to (i) evaluate germination capacity and plant growth capacity of the basaltic parent material, (ii) compare plant growth in parent material with and without compost amendment as compared to potting soil, and (iii) assess soil microbial community composition and functional potential between treatments. The seeds used in this study were sourced from the seed banks of Native Seed Search [10] and were native to the Arizona/Sonora region of Arizona.

2. Results and Discussion

2.1 Germination, plant height, aboveground biomass

Plant characteristics including germination, plant height, and aboveground biomass were monitored for 30 days in a greenhouse (Figure 1). Results are described in Jim and Sengupta [21] with additional statistical testing between all treatments and interpretation presented here.

Figure 1. Greenhouse experimental set-up on Day 10.

Potting soil had highest germination rate (50%) among the bean plants (Figure 2a). Significant germination was not observed between potting soil and parent material with or without amendment for mesquite and grass. Comparison between the LEO parent material and the LEO soil amended with compost for three plant types did not yield significant germination differences. Malfunctioning irrigation drips discovered towards the end of the experiment in one replicate pot each of parent material and parent material amended with compost may explain low germination rates for the bean plants in basalt soil and basalt amended with compost. In conclusion, all three

soil treatments supported germination, with maximum percent germination observed in the potting soil, and overall highest germination of grasses across treatments.

Since one plant per pot was chosen and evaluated for growth during the entire duration of the experiment, the malfunctioning drips did not impact the rest of the observations recorded for plant growth, and above and below ground biomass. As noted in *Jim and Sengupta* [21], significantly higher plant growth was observed in the potting soil as compared to basalt soil and basalt soil amended with compost. The bean and grass plants grew twice the height in the potting soil as compared to basalt with and without amendments (Figure 2b), with grass in potting soil growing to a height of 54 cm while the slowest growing plant was bean in potting soil. Significant aboveground biomass accumulation was observed for bean and grass plants in the potting soil, with lowest biomass accumulation in mesquite across soil treatments [21]. However, it is likely that a period of 30 days may not be sufficient for compost benefits to be visible owing to slow-release of nutrients [22]. Therefore, a longer growth period may be necessary for compost-amendment benefits to be observable.

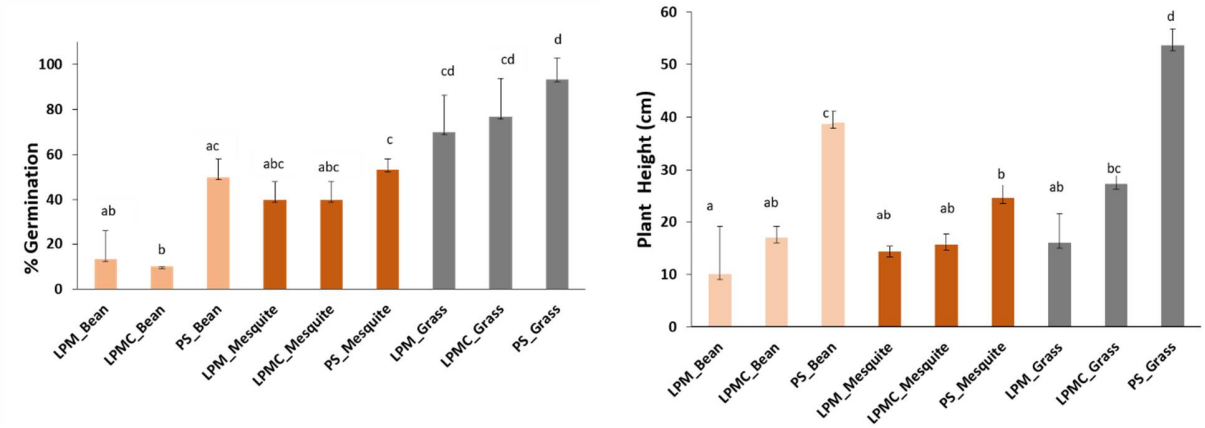
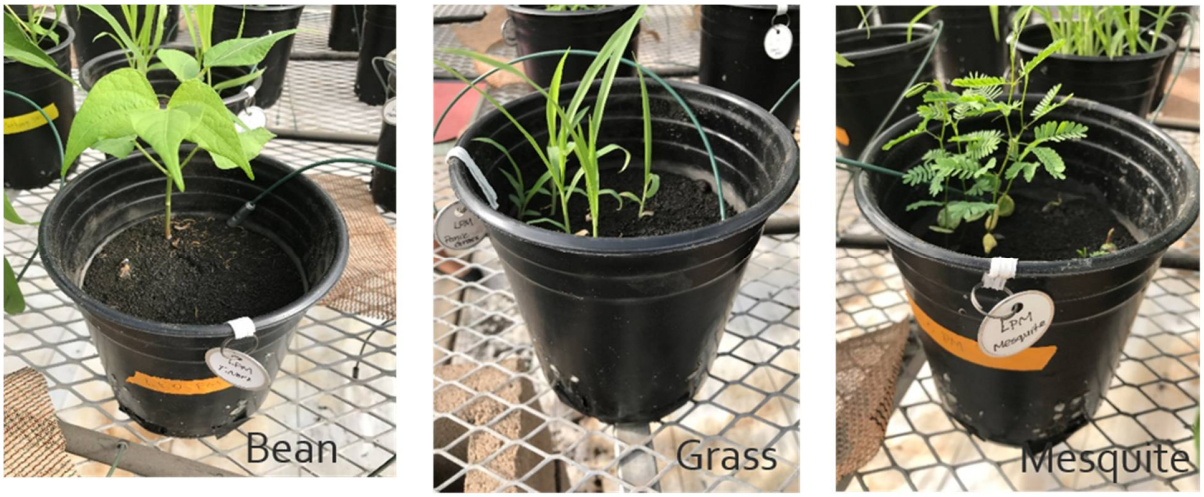


Figure 2. (a) Average percent germination of three replicates, (b) Average plant growth of three replicates. Means not connected by same letter are significantly different at $P<0.05$. Abbreviations:

141 LPM (Leo Parent Material), LPMC (Leo Parent Material + Compost), PS (Potting Soil), and the
142 plant species follow the soil material type.

143 This provides a positive indication of LEO basalt material's ability to support germination
144 and promote plant growth (Figure 3). Additionally, these results show that locally-sourced native
145 seeds can prove to be an effective strategy in vegetating incipient soils that are localized around
146 the native plant source. Moreover, the rapid germination of seeds in the LEO basaltic material can
147 stabilize the soil and facilitate ecosystem succession, as highlighted by Smith et al. [9] who
148 reported rapid revegetation of degraded land by incorporating native plant species.



149

150 **Figure 3.** Growth observed in basalt parent material for all three seed types.

151

152 2.2 Physico-chemical properties of the studied soil materials

153 Physico-chemical data of the treatments as well as irrigation water, parent material, and
154 potting soil is illustrated in Supplementary Figure 1 and, data shared in Supplementary Table1.
155 Soil pH differed between treatments, with pH significantly higher in parent material and parent-
156 material amended with compost as compared to potting soil. A decrease in pH was observed in
157 the basaltic parent material (pH=9.8) in the presence of plant roots (pH=9.4) and compost

amendment (pH=8.5). Bulk density of the potting soil and treatments was significantly lower (average of different plant types = 0.78 g cm^{-3}) than the parent material (average of different plant types = 1.83 g cm^{-3}), and even within the parent material and treatments, significant differences in mean bulk density were also observed. Potting soil and treatments held on average 50 percent moisture and was significantly higher than the parent material soil with or without compost. Carbon and nitrogen estimates were also significantly higher in the potting soil (average of different plant types = 314 ug mg^{-1}) than the parent material and parent material-compost treatments (average of different plant types = 14.24 ug mg^{-1}). The marginal soil characteristic of the parent material (lack of structure, high bulk density, low moisture holding capacity, and low nutrients) as compared to the potting soil, primarily separates the treatments into the two broad groups, as observed in the principal component plot showing variation in soil samples and irrigation water (Figure 4).

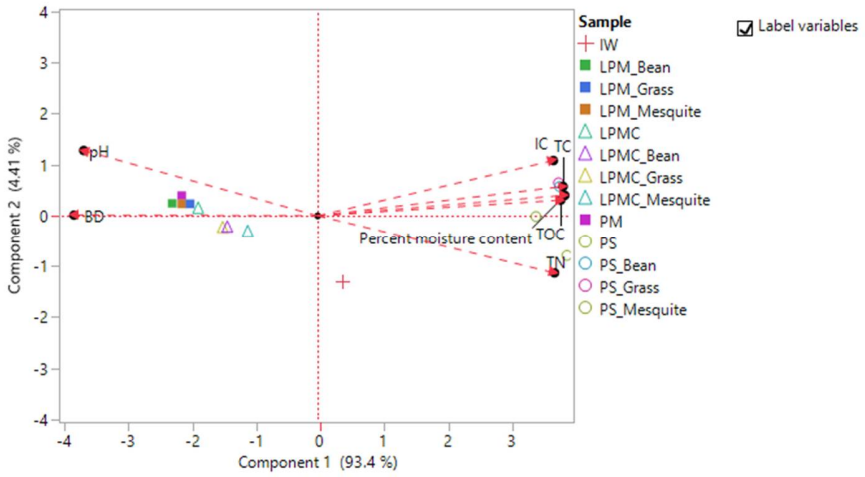


Figure 4. Variation observed in physico-chemical characteristics of triplicate soil-plant treatments and water sample after 30 days, plotted as principle component of variation superimposed by variable loadings. Abbreviations: LPM (Leo Parent Material), LPMC (Leo

Parent Material + Compost), PS (Potting Soil), and the plant species follow the soil material type.

For the LEO parent material, significant differences were not observed for the bare soil and those with plants, while parent material amended with compost had significant increase in carbon (total, organic, inorganic) and nitrogen concentrations for each plant type. It is interesting to note that while plant germination and growth differences did not differ significantly between basalt material with or without compost, the soil characteristics between the two treatments differed significantly. Therefore, soil characteristics likely start to change in these incipient soils within a month when amended with compost though the effects are not parallelly observable in aboveground plant-growth dynamics, suggesting that abiotic shifts in soil characteristics may not have linear observable shifts on plant growth.

2.3 Composition of microbial communities

High-throughput 16S rRNA gene amplicon sequencing was performed to evaluate soil microbial community composition. A total of 5955 Operational Taxonomic Unit (OTUs) were obtained across basalt parent material samples and potting soil samples. The DNA extracts from compost amended LEO soil samples failed to amplify and therefore are absent from the analyses. Despite treating the compost samples with a 5.5 M guanidine thiocyanate (GTC) wash to remove PCR-interfering humic matter [23] and attempting to amplify the samples twice, the compost sample DNA extracts failed to amplify. It is likely that the DNA was coextracted with PCR inhibiting material (either humic acid and/or ions which bind to DNA) in these samples.

Therefore, for this section of the results, the samples were separated into the two broad soil treatments: parent material and potting soil.

A total of 32 phyla were observed across the samples (Supplementary Figure 2). The top ten phyla detected were *Acidobacteria* (0.8-5.7%), *Actinobacteria* (2.6-26%), *Bacteroidetes* (2.1-11.1%), *Chloroflexi* (0.6-2.5%), *Cyanobacteria* (1.1-3.7%), *Firmicutes* (1.4-7.9%), *Gemmatimonadetes* (0.7-2.8%), *Planctomycetes* (0.7-5.0%), *Proteobacteria* (43.9-60.7%), and *Verrucomicrobia* (1.5-16.8%) (Figure 5). *Proteobacteria* were the most abundant phyla in both soil types. The potting soil showed *Actinobacteria* (26%) to be the second most abundant phyla but its abundance reduced in the soil+plus plant (11.1-14.9%). Within the potting soil samples, an increase in Verrucomicrobial OTUs were also observed in the soils with plants (5.8-11.3%) from 1.5% in bare soil. The observed shift in microbial community abundances is likely a result of plant establishment in the potting soils. The abundance of *Verrucomicrobia* (Mesquite > Grass > Bean) showed a similar relative abundance pattern in the basalt soil as in potting soil which could be indicative of plant-specific microbial community response.

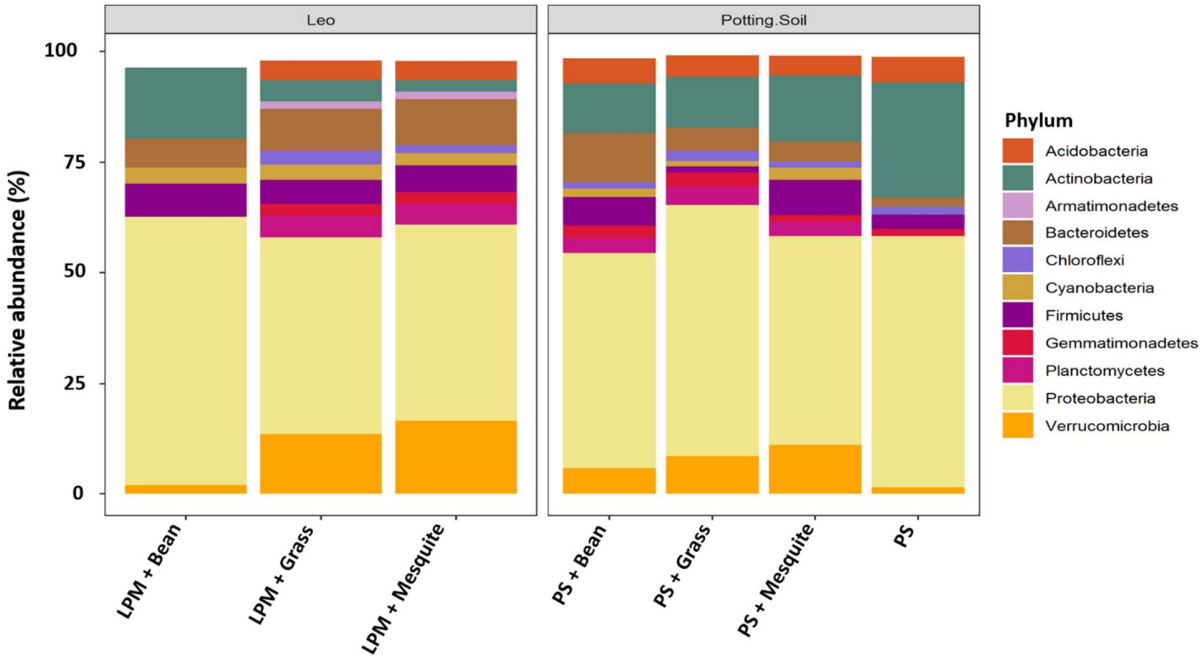


Figure 5. Relative proportions of bacterial community at the phyla level. Phyla representing more than 1% in all samples are summarized and the remaining are indicated as others in the figure. Abbreviations: LPM (Leo Parent Material), PS (Potting Soil), and the plant species follow the soil material type. Sample without plant species name is a control sample.

Furthermore, within the basalt soil type, the bean plant soil had low abundance of *Acidobacteria* (0.8%), *Armatimonadetes* (0.3%), *Chloroflexi* (0.6%), *Gemmatimonadetes* (0.7%), and *Planctomycetes* (0.7%), when compared to the grass and mesquite plant soils. However, *Actinobacteria* was more abundant in the basalt + bean plant soil (16.1%) than in the potting soil + bean plant. The bean plant is a legume and is therefore known to have nitrogen fixation mediated by nitrogen-fixing bacteria in the plant root nodules [24,25]. Interestingly, over the recent years, multiple studies have highlighted the role of many nitrogen-fixing actinobacterial taxa as highlighted in a review by Gtari et al., 2012 [26] as well as the filamentous nature of actinobacterial groups promoting successful colonization of oligtrophic land surfaces[27].

The DNA extracted from the LEO basalt parent material did not have enough number of sequences to pass the sequence analysis quality control in this study. However, as a standalone analysis for visual comparison (Supplementary Figure 3), the parent material community consists of 62% *Bacteroidetes*, followed by 16% *Firmicutes*, and less than 10% *Proteobacteria*. This suggests a dramatic month-long shift in the soil microbial community composition of the basalt soil. Since the seeds were not sterilized before planting, it cannot be ruled out that the seeds may have contributed to the increase in microbial community diversity. We posit that a combination of the parent material, dust and irrigation water input, and seeds provided a heterogeneous initial

composition that increased community richness and introduced an abundance of new taxa in the incipient basalt material.

A comparison of species richness and Shannon's diversity index showed minor differences in the composition of the microbial community (Figure 6). Richness in potting soil samples had a wider range from 478-2593 compared to parent material samples 1686-1985. Potting soil+ bean had the highest richness of 2593 while potting soil + mesquite had the lowest richness of 478. Shannon's indices in parent material samples ranged from 5.0-6.03 versus 4.89-6.07 in potting soils. Richness of microbial communities was not significantly different between the soil types but it was different based on plant types (P=0.03), while Shannon's diversity index did not differ significantly between the soil types as well as plant types.

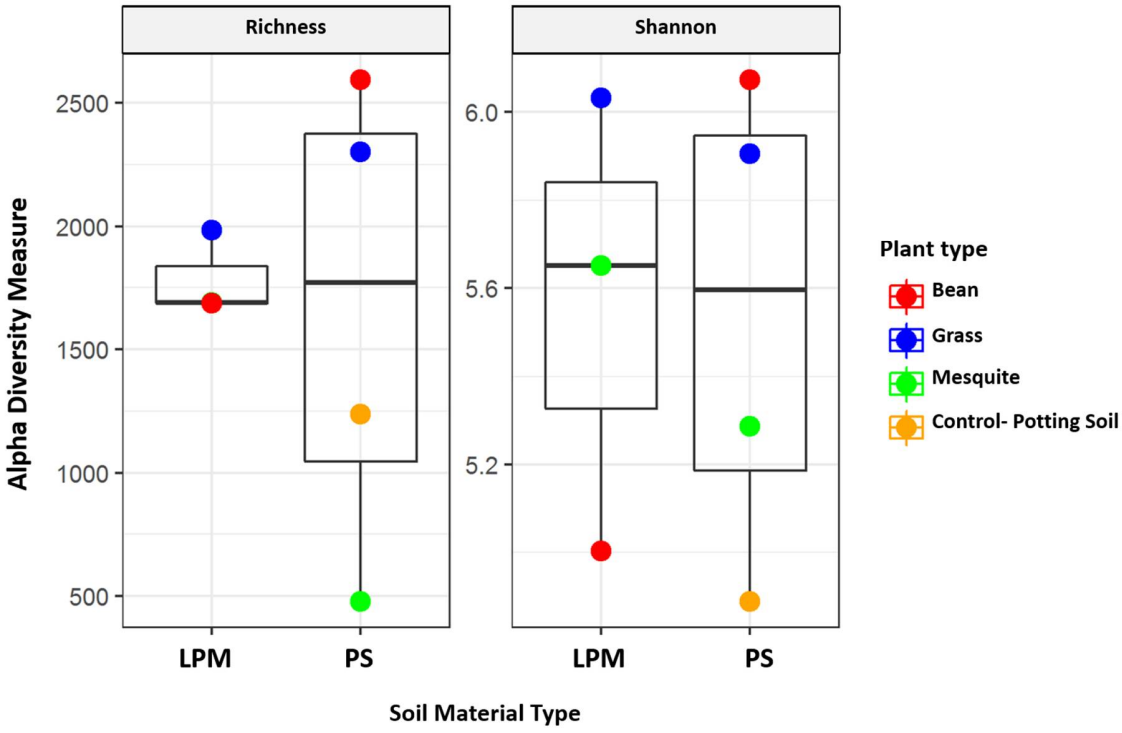


Figure 6. Alpha diversity in the studied soils represented by richness and Shannon's index. The plant type bean, grass, and mesquite are indicated by the colors red, blue, and green.

Potting soil control is represented by the color yellow. The soil material is indicated as LPM (Leo Parent Material) and PS (Potting Soil).

A principal coordinate analysis (PCoA) plot of Bray-Curtis dissimilarity distance-matrix demonstrated the differences in microbial communities between the soil types (Figure 7). The first two PCoA axes together explained 70.4% of the variation in the microbial communities. The samples from each soil type grouped distinctly separate with the control sample of potting soil separated from rest of the potting soil + plant samples. Additionally, PERMANOVA analysis revealed that microbial communities in LEO parent material and potting soil were significantly different (P=0.03) with a R^2 of 0.53 suggesting 53% of the variation in the microbial communities to be explained by the soil material type.

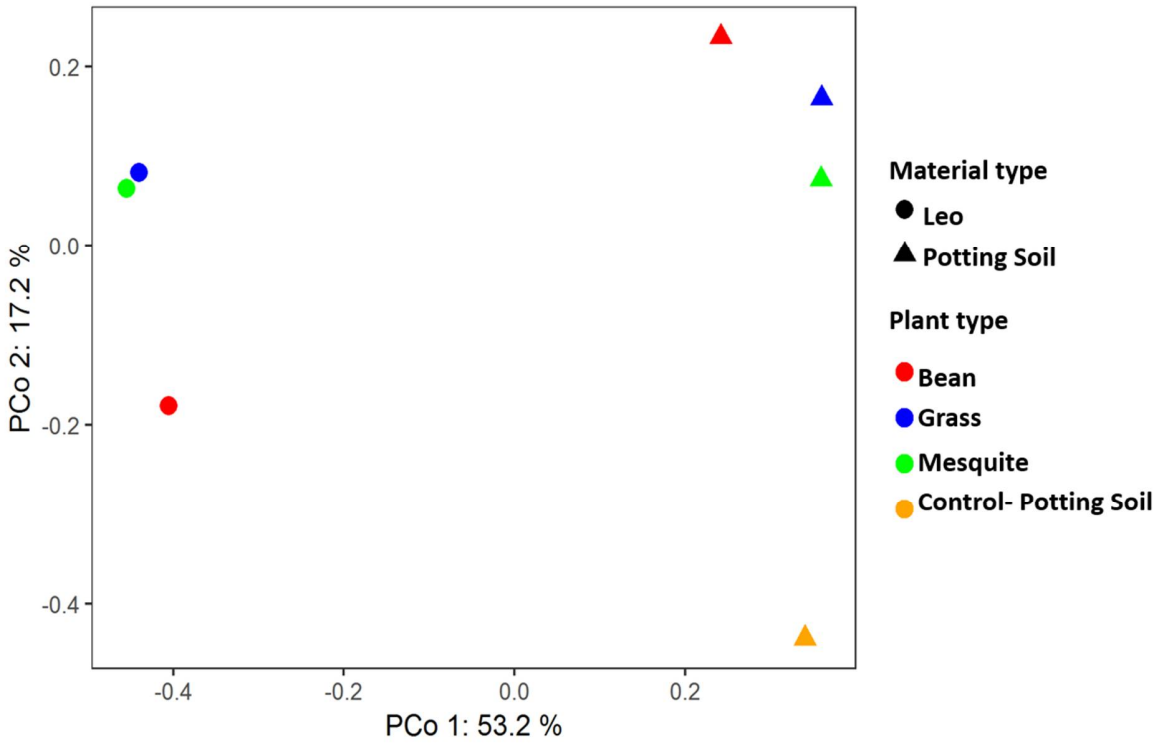


Figure 7. A Principle Co-ordinate Analysis (PCoA) plot based on the Bray–Curtis distance matrix showing similarity of bacterial and archaeal community in the studied soil material type. The first two PCoA axes together explain 70.4% of the variation in the microbial community based on the soil material type.

2.4 Microbial communities and environmental variables

The PERMANOVA and Mantel test were carried out to decipher linkages between soil microbial communities and environmental variables, and between soil microbial communities and plant attributes (Table 1). PERMANOVA as well as the Mantel test demonstrated that majority of the soil factors influenced the microbial communities. Among these soil factors, pH, TC, TIC, TOC, TN, moisture content, bulk density, soil material type exhibited significant ($P < 0.05$) associations with microbial communities. These soil factors that exhibited significant correlations had strong correlation values (r) ranging from 0.71 (TIC) to 0.92 (pH). The soil material type also had a strong significant r value of 0.88, thus indicating that the microbial community shifts were significantly correlated with the soil material type. Plant attributes did not have significant interactions with the soil microbial communities for the duration of the experiment.

Table 1: Correlations between the environmental variables and plant attributes with Bray-Curtis dissimilarity index using a non-parametric multivariate analysis of variance (PERMANOVA) and Mantel test.

	PERMANOVA		Mantel test	
Environmental variables	**R^2**	**P**	**r**	**P**
pH	0.51	**0.003**	0.92	**0.001**
TC	0.53	**0.006**	0.88	**0.008**
TN	0.51	**0.023**	0.91	**0.009**
TOC	0.53	**0.007**	0.86	**0.012**

TIC	0.47	**0.021**	0.71	**0.021**
Moisture content	0.53	**0.022**	0.88	**0.021**
Bulk density	0.49	**0.030**	0.82	**0.017**
Soil material type	0.53	**0.031**	0.88	**0.037**
Plant attributes	**R^2**	**P**	**r**	**P**
Germination rate	0.15	0.441	0.01	0.446
Plant height	0.22	0.218	0.12	0.291
Wet aboveground biomass	0.17	0.443	-0.17	0.701
Dry aboveground biomass	0.18	0.412	-0.15	0.657
Plant type	0.37	0.799	-0.01	0.487

Mantel coefficient (r) ranges from -1 to +1. Value of -1 indicates strong negative correlation, value of +1 indicates strong positive correlation, and 0 means no correlation. Significant values ($P < 0.05$) are in bold.

An observable difference is present in aboveground plant attributes and below-ground microbial community structure shifts. Therefore, as with the abiotic changes in the soil, below-ground biotic changes (in this case microbial community structure) had non-linear associations with plant establishment and growth for the initial time of growth captured in our study. This time-lag between aboveground and belowground shift may be a critical point when evaluating and predicting temporal feedbacks between plant-soil-microbe interactions [28]. These results are useful in informing future directions of our study, where plant establishment and growth in the basalt material can be studied over a longer duration, followed by periodic sampling of soil and plant material to temporally identify linear versus non-linear associations between plant-soil-microbe interactions.

2.5 Predicted functional potential of microbial community

The OTUs were utilized to predict functional potential of the soil microbial community in LEO and potting soil samples. Using FAPROTAX analyses, 59 predicted functions were

296 identified. A subset of 24 functions were further evaluated for their relative profiles in the
297 different soil samples (Figure 8). These functions were chosen to reflect broad carbon
298 fixation/utilization mechanisms (autotrophy versus heterotrophy), and nitrogen cycling
299 metabolisms. Like the microbial community differences, the LEO and potting soils also
300 demonstrated differences in their functional potential. Chemoheterotrophy was the most
301 abundant identified functions within LEO soils while photoheterotrophy and anoxygenic
302 photoautotrophy were higher in the potting soils.

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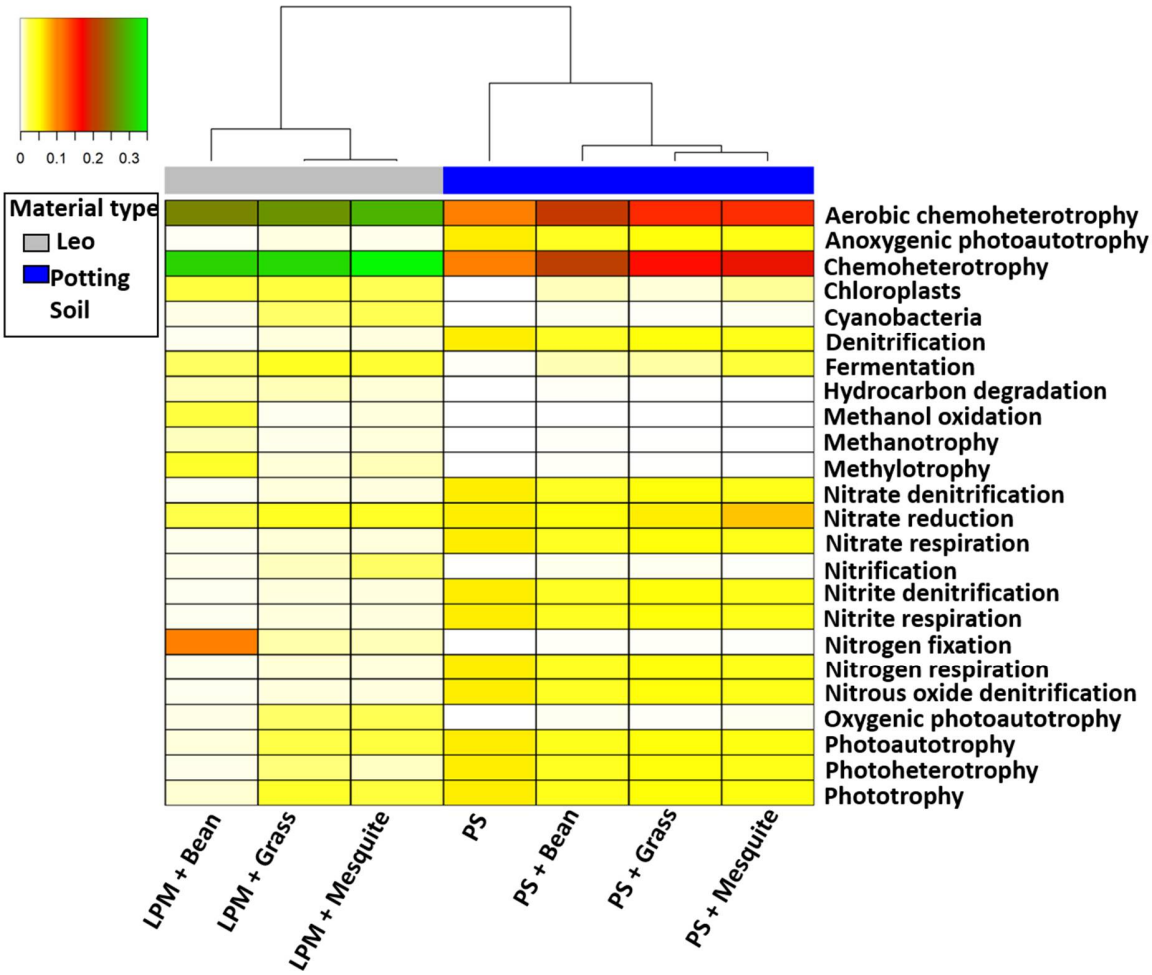

305

306 **Figure 8:** A heatmap showing relative abundance of the selected predicted functional
307 potential of the microbial community using FAPROTAX analysis in the studied soil materials.
308 The relative abundance ranges from 0 to 0.35 and is represented by white (0-0.025), red (0.026-
309 0.1), yellow (0.11-0.21), and green (0.21-0.35), respectively. Abbreviations: LPM (Leo Parent
310 Material), PS (Potting Soil), and the plant species follow the soil material type.
311
312 Majority of the 24 functions were significantly different between the soil types (Table 2)
313 with exception to fermentation, methanol oxidation, methylotrophy, nitrification, and nitrogen
314 fixation. Mantel test showed a strong correlation of the predicted functions with soil type
315 material (r=0.89, P=0.026). The hierarchical cluster grouped the samples according to their soil
316 types indicating the soil type specific functional capacity. The grass and mesquite samples in
317 both LEO parent material and potting soil clustered closely compared to the bean. The basalt soil
318 + bean sample had the highest nitrogen fixation capacity among all samples. In contrast, the
319 potting soils did not have high abundance of nitrogen fixation, but presented higher abundance of
320 other nitrogen cycling pathways (denitrification, reduction, and respiration) as compared to the
321 basalt material soils. Genes associated with fermentation were predicted to be higher in the basalt
322 material which suggests that the basalt material may constitute fermentators which fix carbon
323 [29,30]. While we did not observe high autotrophic predictions for the incipient basalt material,
324 the presence of microbes in this low-carbon soil and the overall increase in organic carbon
325 concentration is evidence of carbon-fixation mechanisms in play. Furthermore, the higher
326 predictive heterotrophic activity in basalt soils could be attributed to the likely diminished
327 autotrophic activity of the *Cyanobacteria* during plant colonization resulting in the availability
328 of fixed plant carbon for heterotrophs [31]. Additionally, we propose a second hypothesis that

329 heterotrophy rates far exceed autotrophy rates in these low-carbon environments. It may be likely
330 that as soon as autotrophs are able to fix carbon and produce by-products, the heterotrophs utilize
331 the fixed carbon and proliferate. The FAPROTAX results should be treated with caution as they
332 do not confirm presence/absence of *in situ* microbial metabolisms. However, these results do
333 serve as a starting point to generate further hypotheses of soil-microbe interactions and metabolic
334 strategies potentially in play in low-carbon versus nutrient-rich soils.
335
336 Table 2. Analysis of Variance showing statistical differences in predicted FAPROTAX functions
337 between soil types.

Predicted Functions	**F**	**P**
Aerobic chemoheterotrophy	28.6	**0.003**
Anoxygenic photoautotrophy	87.8	**0.0002**
Chemoheterotrophy	40.2	**0.001**
Chloroplasts	26.5	**0.004**
Cyanobacteria	7.6	**0.040**
Denitrification	84.1	**0.0003**
Fermentation	4.3	0.094
Hydrocarbon degradation	38.1	**0.002**
Methanol oxidation	2.8	0.157
Methanotrophy	10.7	**0.022**
Methylotrophy	3.9	0.106
Nitrate denitrification	84.1	**0.0003**
Nitrate reduction	12.0	**0.018**
Nitrate respiration	82.8	**0.0003**
Nitrification	4.2	0.096
Nitrite denitrification	84.1	**0.0003**
Nitrite respiration	84.4	**0.0003**
Nitrogen fixation	2.4	0.185
Nitrogen respiration	82.8	**0.0003**
Nitrous oxide denitrification	84.1	**0.0003**
Oxygenic photoautotrophy	7.6	**0.040**
Photoautotrophy	20.3	**0.006**
Photoheterotrophy	45.1	**0.001**
Phototrophy	7.9	**0.037**

338 F-value closer to 1 indicates that means of the two groups are equal. A higher F-value indicates
339 that the means of the two groups are not identical. Significant values ($P < 0.05$) are in bold.

3. Materials and Methods

3.1 Experimental Design

A greenhouse pot experiment was conducted with three seed types (Panic Grass, Mesquite, Common Bean) and three soil materials: Basaltic parent material from the LEO experiment (LPM), LEO parent material + 20% w/w commercially available compost (LPMC), and commercially available potting soil (PS). The total carbon, organic carbon, and nitrogen concentrations in the compost material was 138 ± 7 µg g^{-1}, 120 ± 5 µg g^{-1}, and 3.0 ± 0.56 µg g^{-1} respectively; while potting soil recorded 294 ± µg g^{-1}, 213 ± 3.0 µg g^{-1}, and 13.34 ± 1.0 µg g^{-1} respectively. Compost was added to evaluate the effect of natural amendment on LPM's capacity to support plant growth while potting soil served as a positive control. Each seed type-soil material combination (n=27) was set-up in randomized triplicate 1-gallon plastic pots with 2.1 kg LPM, 2.1 kg (1.68 kg LPM + 0.42 kg compost) LPMC, and 0.6 kg PS, and ten seeds sown per pot. The drip irrigation system was controlled at three one-minute events a day at 8:30 a.m., 12:00 p.m., and 4:30 p.m. respectively; seven days a week totaling 720 ml/day/pot of tap water and pots monitored for 30 days (June 24th-July 24^{th,} 2017).

3.2 Seed information

Detailed seed information and sowing is provided in a preliminary publication by Jim and Sengupta [21]. Briefly, germination and growth response of three seeds types were studied: *Panicum Sonorum* (Panic Grass), *Prosopis spp.* (Mesquite) and *Phaseolus vulgaris* '*Tarahumara Norteño*' (Common Bean). Panic grass was cultivated in the Arizona and Sonora region four thousand years ago. *Prosopis* spp, or Mesquite grows in arid and semi-arid environments such

as, deserts, woodlands, floodplains, grasslands, and shrublands, have deep tap roots with leaves adapted to reduce water loss. *Tarahumara Norteño* bean (also known as the common bean), originated in the Tarahumara area of the Chihuahua region in Mexico and has been widely cultivated by Native American farmers throughout the Southwest [10,32]. Common beans are known to grow in semi-tropical regions and hosts nitrogen-fixing bacteria in its root nodules. The three seed types typically grow in arid to semi-arid environments, are heat tolerant, can retain water, and have short germination periods. The grass and bean seeds were sourced from Native Seeds, Tucson, AZ and mesquite seeds were sourced from Desert Nursery, Phoenix, AZ.

3.3 Plant and soil measurements

Percent germination was calculated after ten days. One plant per pot was marked and monitored continuously, with height measurements taken once every week. At the end of the experiment, the marked plant was harvested for wet and dry aboveground biomass measurement as per protocol outlined in Jim and Sengupta [21]. Bulk density (BD) cores were collected using metallic cores of height 2.9 cm and diameter 5.3 cm. Bulk soil samples close to the roots were also collected for geochemical and microbiological analyses, stored on ice, and brought back to the lab for processing. Half of the bulk samples were air-dried and sieved for pH, and carbon and nitrogen measurements, while the other half was frozen at -80 °C. Percent moisture was measured after drying field-moist soils at 105 °C for 24 hrs. Total carbon (TC), organic (TOC), inorganic carbon (TIC), total nitrogen (TN) were measured using US EPA method 415.3, whereas pH was measured using the US EPA method 150.2.

3.4 Soil microbial community analysis

Soil microbial DNA from the frozen soils was extracted and sent for sequencing to the University of Arizona Genetics Core (UAGC; Tucson, AZ, USA). Sequence data was analyzed as per protocols highlighted in Sengupta et al. 2018 [20]. Briefly, paired-end sequencing (2x150 bp) was performed on the bacterial and archaeal 16S rRNA gene V4 (515F-806R primers) hypervariable region using the Illumina MiSeq platform (Illumina, CA, USA) [33]. All of the sequencing procedures, including the construction of Illumina sequencing library, were performed using the protocol previously published [34] with modifications [35]. Illumina MiSeq v2 (300 bp) chemistry was used for sequencing and was performed on the Illumina MiSeq (SN M02149, with the MiSeq Control Software v 2.5.0.5) at the UAGC following their standard protocols. The UAGC provided standard Illumina quality control, base-calling, demultiplexing, adaptor removal, and conversion to FastQ format. Raw sequence data are submitted to NCBI's Sequence Read Archive SUB4001574, ProjectID PRJNA464263.

Paired-end sequence merging, barcode removal, quality filtering, singleton-sequence removal, chimera checking and removal, and open-reference Operational Taxonomic Unit (OTU) picking were conducted using default parameters unless otherwise specified in Qiime v 1.9.1 [33]. A minimum of 20 base-overlap was specified for joining the paired reads to give an average sequence length of 253 base pairs. A summary of the sequences, post-merging and quality filtering, was performed using mothur (v 1.25) (26), OTU picking was done using UCLUST [36], and sequence alignment was performed with PyNAST (28). Clustering was done with Greengenes database at 97% sequence similarity [37], chimera were removed with Chimera Slayer [38], taxonomy was assigned with RDP Classifier [39], tree building was completed with FastTree [40], and comparative diversity calculations were done with UniFrac [41]. OTUs that were observed only once after chimera filtering were removed. All data files generated from

QIIME workflow were imported into *R* environment program [42] for alpha and beta diversity estimation and visualization using *Phyloseq* [43].

3.5 Functional annotation of sequence data

The 16S rRNA amplicon gene sequences were used to infer metabolic traits from phylogeny using the tool Functional Annotation of Prokaryotic Taxa (FAPROTAX) [44]. FAPROTAX facilitates interpretation of microbial functional profiles from 16S rRNA bacterial and archaeal sequence data, based on available literature of cultured representatives. Briefly, an OTU is associated with a particular metabolic function if all cultured representatives of that OTU are reported to exhibit that function. For example, if all cultured representatives of a genus have been identified as nitrifiers, FAPROTAX assumes all uncultured members to be nitrifiers as well. This approach was well suited to our study given the complexity of soil environments, and large portion of soil microbes remain uncultured.

3.6 Data Analyses

Percent germination, plant height, aboveground wet and dry biomass, pH, bulk density, carbon (total, organic, and inorganic), total nitrogen, and moisture content were statistically analyzed using JMP® 13.0. Significant differences of mean were determined by one-way ANOVA ($P < 0.05$) followed by pairwise comparisons of the means of each soil material/plant group using Tukey's HSD with significance levels at $P < 0.05$. Sequences were evaluated for alpha (Richness and Shannon's Index), and beta diversity metrics (Bray-Curtis) analyzed with Principal Coordinate analysis (PCoA). To visualize predicted functional differences between the soil types, a heatmap was generated using *gplots* (36) package in R. Additionally, a hierarchical

cluster was generated using the average linkage method. To determine the dissimilarity in the microbial community structures and the soil physicochemical properties together with plant measurements, a non-parametric multivariate analysis of variance (PERMANOVA) was performed based on the Bray–Curtis dissimilarity. Additionally, Mantel tests were performed to determine correlations between the soil microbial communities and the environmental factors. PERMANOVA and Mantel's test analyses were carried out in R (v.3.5) using the package *vegan* [45] and *ade4* [46], respectively. ANOVA analysis was conducted using relative abundance of OTUs classified as specific functional guilds using FAPROTAX. The F-value was used to evaluate the ratio of mean square values of the samples separated into the two soil types.

4. Conclusion

The conceptual framework of Land Degradation Neutrality is based on balancing current soil degradation by reversing past degradation via restoration, rehabilitation, and reclamation. The ability to restore/rehabilitate/reclaim marginal soils is crucial to this framework. In this study, plants native to the geographical area and climate of a marginal incipient basaltic soil material, with and without organic amendment, were evaluated for their germination and growth attributes. Comparisons of soil microbial community and plant attributes were made between the incipient soil and a commercially available potting soil. The results show that marginal incipient basaltic soil has the ability to support native plant growth and that distinct soil microbial communities develop in these soils alongside plant establishment. Furthermore, nonlinear associations between abiotic shifts in soil characteristic, microbial community compositional changes, and plant growth parameters exhibited nonlinear associations. A direct outcome of this study is being applied to current experiments being conducted at the Landscape Evolution Observatory to establish plants and monitor the co-evolving hydrobiogeochemical signatures in

the incipient landscapes. As a future direction, we propose detailed experiments with longer growth periods and different combinations of marginal soils with and without amendments to evaluate the capacity of such soils to support and sustain plant growth. Additionally, an approach of sourcing and using native seeds can be a collaborative exercise between scientists and social scientists to involve and evaluate native cultures' knowledge base to answer modern-day sustainability issues.

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Supplementary Materials

Supplementary Table S1 Raw data (soil characterization and plant attributes)

Supplementary Figure S1. Physico-chemical characteristics of irrigation water, parent material, potting soil, and treatments. Values are means. Means not connected by same letter are significantly different at $P < 0.05$ (one-way ANOVA,Tukey's test)

Supplementary Figure S2. Relative abundance of microbial community at the phyla level**.** Abbr. LPM (Leo Parent Material), PS (Potting Soil)

Supplementary Figure S3. Relative abundance of the incipient basaltic parent material

Supplementary Figure S4: Relative abundance of bacterial and archaeal community at the class level. A total of 85 classes were identified. Abbreviations: LPM (Leo Parent Material), PS (Potting Soil), and the plant species follow the soil material type. Sample without plant species name is a control sample.

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Author Contributions

Conceptualization, AS; Methodology, AS and AJ.; Formal Analysis, AS, AJ, PK, Investigation, AS, PK, AJ; Resources, PT, RM; Data Curation, AS, PK; Writing – Original Draft Preparation, AS, PK; Writing – Review & Editing, AS, AJ, PT; Visualization, AS, PK, AJ.; Supervision, AS; Project Administration, AS; Funding Acquisition, PT, RM